

Effect of Gravity on the Colonial Morphology of Staphylococci in Soft Agar

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After horizontal rotation on a clinostat at 1 rev/min, subsurface colonies of staphylococci in soft agar were compact and spherical; nonrotated colonies were diffuse and elongated.

It has been established that the subsurface colonial morphology of staphylococci is dependent upon the concentration of agar in the medium (1-3, 5). At agar concentrations of 0.18% or less, generally referred to as soft agar, elongated diffuse colonies were produced; at higher agar concentrations, compact spherical colonies developed (Fig. 1). Alami et al. (1, 2), Finkelstein and Sulkin (3), and Yoshida and Ekstedt (5) noted that when fibrinogen, serum, or coagulable plasma were incorporated in soft agar, the colonies of staphylococci positive for coagulase or clumping factor were compact and spherical. On the other hand, colonies negative for these factors were diffuse and elongated. Finkelstein and Sulkin (3) speculated that the action of coagulase on the plasma resulted in an increased viscosity in the immediate colonial environment which, in turn, influenced the morphology of the developing colony.

We have been able to demonstrate compact spherical colonies of *Staphylococcus aureus* in soft agar medium by the horizontal rotation of cultures in a clinostat which produced a uniform gravity field. These findings confirmed the statement by Alami et al. (2) that "elongation of staphylococci colonies in basal soft agar is due to gravity effect," and it also demonstrated that such colony morphology could be altered by changes in the gravity field.

A coagulase-positive culture of *S. aureus*, isolated from the integrated life-support system test chamber at the Langley Research Center, or ATCC strain 12600 were used in these studies. Approximately 0.75 ml of inoculated soft agar containing 3.0% Trypticase Soy Broth (BBL) and 0.16% Agar-Agar (BBL) was transferred to sterile 5 by 50 mm test tubes which were layered with 5.0% agar to prevent slippage during rota-

tion on the clinostat. Each tube had a final concentration of about 4 to 6 colony forming units of *S. aureus*. One such culture was rotated in the horizontal position in a clinostat operating at 1.13 rev/min. Appropriate controls were maintained by placing nonrotated cultures in the vertical and horizontal positions. Both still and time-lapse photography were used to record the development of colony morphology in the clinostat and control cultures. The rotating clinostat and camera were synchronized so that the same field would be photographed without interrupting rotation of the clinostat. For most experiments, the cultures were incubated at 35 C, whereas the time-lapse photographic studies were conducted at room temperature. No differences in morphology were noted between the two temperature conditions.

The initial morphology of the control colonies in agar concentrations less than 0.20% was that of an oblate spheroid. After further incubation, there was downward, diffuse streaming of cells from the spheroid to form a gravity-oriented tail which continued to increase in length for about 2 weeks. After this time, a dumbbell-type of colony morphology developed with bands or zones of heavier growth scattered between the two terminal areas of growth. After rotation of *S. aureus* in a clinostat at 1.13 rev/min, the diffuse, feathery colony did not develop. Instead (Fig. 2), after rotation for 18 hr, compact spheroid colonies were observed which appeared to be larger and not as tightly compact or spherical as colonies which developed in agar concentrations greater than 0.6%. In the interrupted clinostat studies, the compact spheroid colonies which had developed after rotation were now incubated in a stationary horizontal position. After 72 hr, the spheroid mass of cells elongated on a gravity-oriented axis, and the diffuse area of cells which appeared gave

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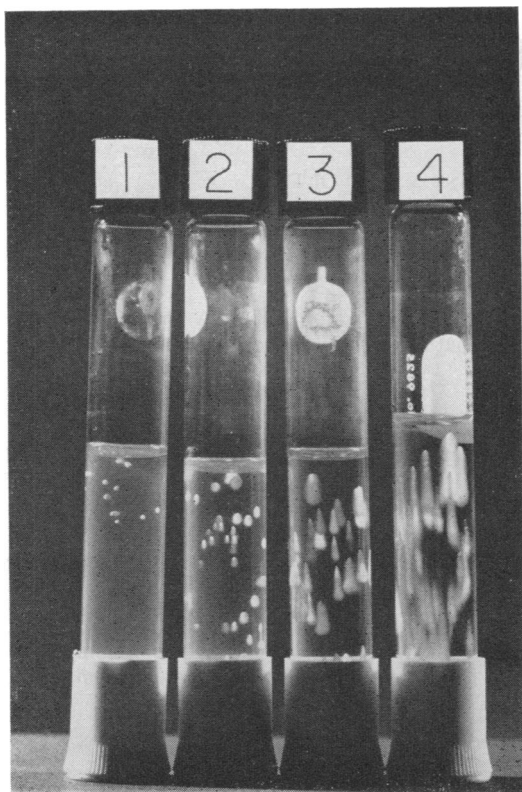


FIG. 1. Influence of agar concentration on the colonial morphology of *S. aureus* after incubation for 24 hr at 35 C. (1) Compact spherical colonies, 0.54% agar. (2) Initial tendency toward colonial elongation, 0.35% agar. (3) Further elongation leading to a teardrop shape, 0.27% agar. (4) Elongated diffuse colony in soft agar, 0.18%.

the colony a comet-like appearance. An additional 48 hr of rotation induced a slight reversal to the spheroid shape in the elongated mass, but little or no change was observed in the diffuse region. At this time, one can only speculate on the relationship between these observations and the effect of a reduced gravity field in space on the growth of microorganisms in vitro, as well as in vivo. It is of interest, however, that in data from an automated laboratory spacecraft, Biosatellite II, Johnson and Tibbitts (4) confirmed the hypothesis that orbital weightlessness produced results with the pepper plant which were similar to studies conducted with a ground-based rotating clinostat.

LITERATURE CITED

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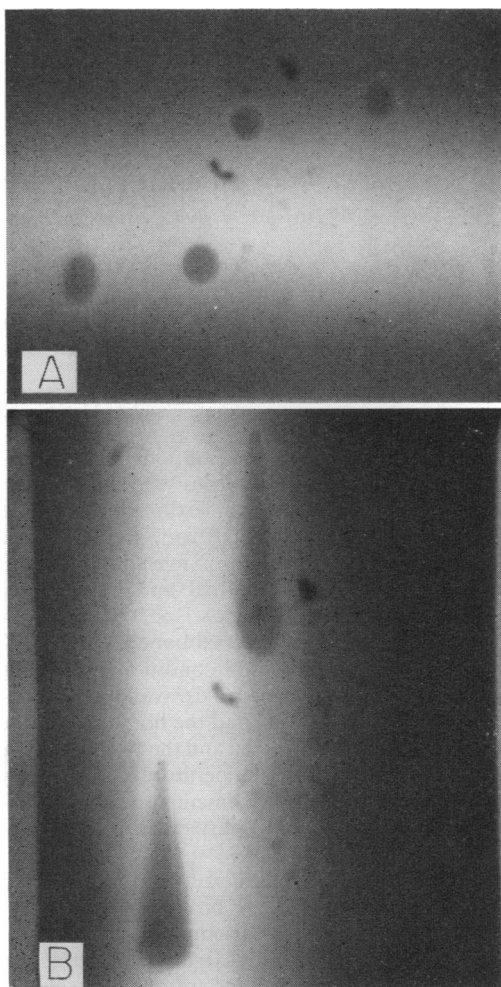


FIG. 2. Compact spherical colonies of *S. aureus* in 0.16% agar after rotation for 18 hr in a clinostat at 1.13 rev/min (A), and diffuse, feathery growth of non-rotated control (B).

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